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A STORAGE ROT OF POTATOES CAUSED BY A FLUORESCENT ORGANISM RESEMBLING *PSEUDOMONAS FLUORESCENS* (FLÜGGE) MIGULA[1]

BY E. H. GARRARD[2]

Abstract

A storage rot of potatoes caused by a fluorescent organism is described. Artificial inoculations produced rapid blackening and rotting of tubers at temperatures below 20° C. Most extensive rot was produced at 5° C. After successive transfers on culture media, the organism lost its pathogenicity within six months. A description of the organism is given, showing its close resemblance to *Pseudomonas fluorescens*.

Introduction

Fruit and vegetable rots caused by *Pseudomonas fluorescens* (Flügge) Migula, or by closely related fluorescent organisms, have been reported on various occasions. Chief among these is a storage rot of celery reported by Barlow (4) in which the celery was attacked by two strains of *P. fluorescens* after its resistance had been reduced by low temperature. Laurent (7) recorded a stem rot of tomatoes caused by *Bacterium fluorescens liquefaciens* and Linhart (8) claimed that the same organism was one of the causes of rot of beet seedlings. According to Clara (3) the organism is weakly pathogenic for the fruit of Keiffer pear. Several examples of potato rot caused by fluorescent organisms resembling *P. fluorescens* have been recorded. Lacey (6) isolated a strain causing a ring disease of potatoes in which a brown rot of the vascular system and slight necrosis of the pith were produced. An organism closely resembling *P. fluorescens* was isolated from a diseased tuber by Israilsky and Runov (5). Schuster (9) isolated *Bacterium xanthochlorum* (Schuster) Bergey *et al.*, a green fluorescent organism bearing a close relationship to *P. fluorescens*, which caused a storage rot of potatoes. No recent reports of storage rot of potatoes caused by *P. fluorescens* have been observed, and in view of the distinctive type of rot, the following case is recorded.

Appearance of Rot

Diseased potatoes were received for examination in March, 1944, from the Newmarket district, north of Toronto. They were grown during the preceding year in Victory gardens and had been stored under supposedly ideal conditions.

[1] *Manuscript received January 19, 1945.*
Contribution from the Department of Bacteriology, Ontario Agricultural College, Guelph, Ont.

[2] *Professor and Head of Department of Bacteriology.*

The rot in the tubers was not noticed until they were cut open for cooking. According to information received, no diseased conditions were noticed in the plants, nor were conditions such that any rot would be expected.

Four separate lots of potatoes were received from different gardeners; in each lot the symptoms were practically identical. The tubers were slightly flabby, and varied from those that showed no exterior signs of disease to others that showed discoloured areas on the surface. A few of the potatoes showed evidence of scab, but none showed cracks. When cut open, striking symptoms of a hard, black rot were exhibited, entirely unsuspected by external appearance. There was a definite vascular decay of a corky nature, brown to black in colour and, in some, cracks and cavities were present. Brown to black areas, generally radiating toward the outside, were found in the centre of the tuber; in more diseased tubers cavities were in evidence. Fig. 1 shows tubers infected to varying degrees and illustrates the black centres and vascular infection. In Fig. 2, the black central areas have spread to the vascular ring and cavities are noticeable. When freshly cut, the diseased tubers were quite hard with little exudate and no ropiness. The odour was not pronounced. Cut surfaces of tubers not seriously affected first turned pink then sooty black in colour; this blackening was hastened by ice-box temperatures. When diseased potatoes were exposed to room temperature, progressive rotting was not nearly so rapid or pronounced as when they were kept at 5° C.

Isolation of Causal Organism

In some respects, the symptoms resembled those of bacterial ring rot, but repeated platings on Burkholder's medium, as described by Skaptason (10) failed to reveal the presence of the ring rot organism. Plates poured with nutrient agar, however, produced colonies resembling those of *P. fluorescens*. In one instance, no other type of colony was found on the plates. Such colonies were obtained from both the vascular and central areas. They resembled the flat, greyish-white, circular to lobate colonies of *P. fluorescens*, with glistening appearance and yellowish-green fluorescence in the surrounding medium. Numerous colonies were picked and transferred to slants and inoculations were made on sterile slices of potatoes in deep Petri dishes. These were incubated at temperatures of 25°, 18°, and 5° C. Little expectation was held that diseased conditions would be produced as the isolated organism was thought to be entirely saprophytic.

After incubation for 24 hr., it was observed that the inoculated slices of tubers were turning brown to black at temperatures of 18° C. and 5° C. but not at 25° C. At 18° C. the diseased areas were brown, while at 5° C. the

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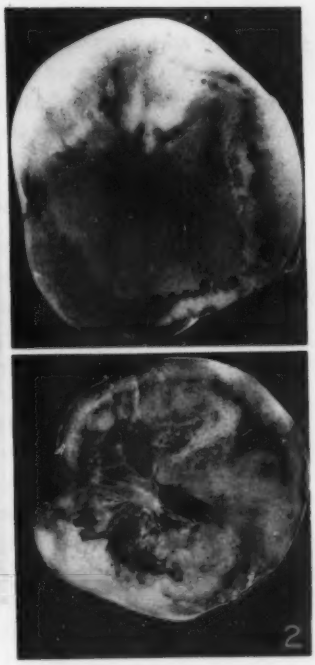
EXPLANATION OF PLATE I

FIGS. 1 to 4. *Potatoes naturally and artificially infected.* 1 AND 2. *Sections of naturally infected tubers illustrating black central diseased areas, vascular infection, and cavities.* 3. *Artificially inoculated sections showing blackening and rotting after one week at 5° C.* 4. *Appearance of artificially inoculated tubers after six weeks at 5° C.*

1


2

3

4

colour was black and greater areas were affected. At this latter temperature, the rot had penetrated to a depth of 2 to 3 mm. After incubation for one week at 25° C., a slight browning was exhibited but no rot had developed, while at 5° C. the rot was general and cracks had appeared. From these inoculated tubers the organism was re-isolated and new tubers inoculated; the same results were obtained.

Artificial Inoculations

As shown in Fig. 3, the necrosis resulting from artificial inoculation differed somewhat from that in potatoes naturally infected. A brown area around the site of inoculation was followed by blackening and spreading until, in most cases, the whole surface of the tuber became infected. After one week the rot had penetrated to a depth of approximately 6 mm. below the surface; this blackening was preceded by a greenish fluorescence that permeated the whole tuber. In each case the blackening and rotting were more pronounced and rapid at ice-box temperature (5° C.) and at no time during succeeding experiments was rotting produced at 25° C. on uncooked potatoes. At 5° C. the inoculated tubers presented at first a hard, firm rot, but eventually they became soft and of a butter-like consistency. Microscopical examination of the diseased tissue showed the starch grains to be unaffected. After six weeks' incubation at 5° C. the remnants of the tubers were shrunken with large cavities in the centre and cracks in the marginal areas. This is shown in Fig. 4.

Sound, whole tubers were next inoculated. These were washed, immersed in mercuric chloride (1 : 1000) for one hour, re-washed with sterile water and stabbed with a sterile needle. Inoculum from fresh cultures was introduced down the line of stab and the opening sealed with melted paraffin wax. Other tubers were cut almost in half with a sterile knife, inoculum was deposited on one or both of the interior surfaces, and the halves closed and sealed with wax. The tubers were incubated at temperatures of 25°, 18°, and 5° C. and examined after two weeks. At 25° C. the tubers showed a slight browning, with a hard, almost metallic surface but there was no evidence of rot. At 18° C. the stabbed tubers were brown along the line of stab and corky cavities were formed. The cut tubers were brown to black with a hard rot that had spread over more than half the surfaces. The most marked effect was shown in those tubers incubated at 5° C. where blackening and extensive rot was most pronounced. These are shown in Fig. 5 (A, B, and C).

Experiments with boiled potatoes, inoculated and incubated at the same temperatures, showed entirely different results. At temperatures of 18° C. and 5° C. there was no action and the organism grew on the surface as on a slant. At 25° C., however, the cooked tubers were rapidly broken down with profuse gas production and a pronounced odour.

Inoculation by Submersion

In order to determine if the organism could penetrate the unbroken skin of the tuber, various new and old sound tubers were thoroughly washed in frequent changes of sterile water and submerged in heavy broth and water suspensions of the organism. They were incubated at the three temperatures previously mentioned, for periods of time ranging from 10 to 72 hr. A number of tubers were planted in soil and others were held at various temperatures for more than two weeks. The planted tubers were allowed to grow to a height of 6 in. before being examined. Examination of all tubers submerged in bacterial suspensions failed to show any signs of rotting. Inoculated cut surfaces as controls were blackened and rotted in 24 hr. at 5° C.

Inoculation of Plants and Varieties of Potatoes

Sound tubers were grown in pots and when approximately 10 in. high, they were inoculated under forced humid conditions, by spraying the leaves and by making needle injections into the base of the stems. The plants were kept for a month and examined daily, but no rot developed in leaves or stems.

To determine if there was any difference in varietal resistance to the organism, 10 varieties of potatoes were obtained. These consisted of: Houma, Warba, 320-302, Columbia Russett, Green Mountain, Chippewa, Irish Cobbler, Bliss Triumph, Katahdin, and Dooley. These tubers were surface sterilized, inoculated by the methods previously described and incubated at temperatures of 25°, 18°, and 5° C. At the end of two weeks they were examined and found to be equally affected. The rot was pronounced at 5° C. and absent at 25° C.

Cut surfaces of carrots and turnips were inoculated and incubated at the same temperatures but after two weeks neither blackening nor rotting had developed.

Description of the Organism

It was thought that the isolated organism would prove to be a definite plant pathogen, but subsequent pure culture work revealed its resemblance to *P. fluorescens*. It also closely resembled *Bacterium xanthochlorum* and Lacey's 221 strain (6). The main difference between the organism and others was its ability to grow and produce rot at low temperatures. The following is a description of the organism.

A medium sized rod, measuring 0.7–0.9 × 1.5–3.0 μ, motile by one to three polar flagella; no spores; Gram negative; grouped singly and in pairs. Aerobic; nutrient agar colonies—greyish-white, opalescent, circular, flat, and

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EXPLANATION OF PLATE II

FIG. 5. *Effect of temperature on artificially inoculated tubers. On the left, whole tuber stabbed; centre and right, tubers cut, inoculated, and sealed. A. Two weeks at 25° C.; browning but no rot. B. Two weeks at 18° C.; blackening and shallow rot. C. Two weeks at 5° C.; blackening and extensive rot.*

A

B

C

FIG. 5

glistening, yellowish-green colouration produced in the medium; gelatine—rapidly liquefied, saccate to infundibuliform with no sediment or fluorescence; agar slant—filiform, smooth, glistening, green colouration in the medium; pronounced disagreeable odour. Beef broth—turbid with fragile pellicle and slight sediment, little or no fluorescence; litmus milk—no coagulation, alkaline and rapidly clearing; nitrates reduced; no indol; no hydrogen sulphide; no starch hydrolysis; lipolytic; no growth in Cohn's solution, prolific growth in Uschinsky's medium with pronounced green colouration; oxidase positive; sodium citrate positive. No gas but acid from dextrose, galactose, levulose, mannose, arabinose, xylose, and mannite; organic acids fermented—malic, acetic, lactic, citric, and succinic. No growth at 37° C., optimum 25° C., and minimum 2° C.; pH range—5.0 to 8.3. Thermal death point about 62° C. Dies within 10 days in dried condition at room temperature and within 30 days at 5° C.

In the original fermentation tests, using ordinary sugar broth with indicator, no fermentation was detected. By using the medium of Ayers, Rupp, and Johnson (1), which is particularly useful for organisms producing an alkaline reaction, acid from the above sugars was detected in less than a week. For organic acid fermentation, the synthetic organic acid medium advised by the same authors was used.

Decreasing Pathogenicity

When first isolated, the organism was particularly pathogenic for potatoes, causing blackening and rotting in 24 hr. at temperatures below 20° C. On one occasion, transfers made in error on tryptone agar slants and inoculated on cut surfaces of tubers produced brown to black areas in two hours at temperatures of 18° C. and 5° C. The organism was periodically transferred two to three times per month and it became apparent that after the 15th transfer it was losing its ability to produce rot in tubers. On numerous occasions throughout these experiments it was thought that the culture might be a mixed one and that the strain pathogenic for tubers was becoming masked or outgrown by *P. fluorescens*. Although single cell technique was not adopted, repeated platings in high dilutions from cultures and inoculated potatoes, and careful microscopical examinations of the colonies, failed to reveal the fact that more than one type of organism was being cultured.

After the 21st transfer on nutrient agar, the organism failed altogether to produce blackening or rotting of tubers incubated at any temperature. Fresh isolations from previously inoculated tubers produced rot for a time and then they too gradually lost their pathogenicity. All attempts to restore its pathogenicity so far have failed.

Discussion

In view of the fact that this organism is practically indistinguishable from *P. fluorescens* on culture, and that it lost its pathogenicity for potatoes after approximately six months, there seemed no justification for classifying it as a separate plant pathogen. It is known that apart from pathogenicity, there

are few distinguishing characteristics between the saprophytic fluorescent types and the fluorescent plant pathogens, and the writer agrees with Burkholder (2) that very slight cultural differences among these fluorescent organisms hardly justify the creation of new species. There seems little doubt that this isolated organism belongs to Subgroup II mentioned by Clara (3), which consists of fluorescent non-plant pathogens and those weakly parasitic. The organism resembles, in many respects, Lacey's strain 221 (6), except that her isolate caused extensive rot at 30° C.; her organism differed also in hydrolysing starch and sucrose, though weakly, and did not produce acid from mannite. *Bacterium xanthochlorum*, which also it resembles to some extent, differs from it in liquefying gelatine slowly, in producing indol and hydrogen sulphide and in having a maximum growth temperature of 44° C. With the exception of its inability to grow at 37° C. this isolated fluorescent organism very closely resembles most descriptions of *P. fluorescens*. No suggestions are offered as to the method whereby the potatoes originally became affected. It is generally recognized that *P. fluorescens* may attack fruit and vegetables if they have become weakened—particularly by prolonged exposures to low temperatures. As these potatoes were stored during the winter months under home-made conditions, it is possible that their resistance was so lowered as to allow attack by this strain of organism that was able to cause rotting at a temperature approximately that used for storing potatoes.

In all probability, these few cases represent only isolated incidences, and a rot produced by this type of organism may play a very minor part in storage rot of potatoes. However, the fact that a strain of *P. fluorescens* can produce a black rot of tubers at storage temperatures indicates possibilities that should not be ignored.

Acknowledgments

The author wishes to express his gratitude to Dr. J. H. L. Truscott, Chief, Horticultural Research, Department of Horticulture, Ontario Agricultural College, and Dr. J. D. MacLachlan, Department of Botany, Ontario Agricultural College, for their constructive criticism and to N. M. Parks, Supervisor, Potato Investigations, and J. W. Scannell, District Inspector, Potato Certification, for their interest and kindness in obtaining diseased and sound varieties of potatoes.

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LIBERATION OF GROWTH STIMULATING MATERIALS BY ROOTING *SALIX* CUTTINGS[1]

By N. H. Grace[2]

Abstract

Physiologically active substances were liberated by rooting willow cuttings and retained by the solution or sand media in which rooting had occurred. Subsequent rooting of willow cuttings in such media affected the number and particularly the length of roots. Stimulation of root growth on dormant cuttings occurred while the effects on non-dormant material were predominantly injurious. Stimulation or inhibition was related to the concentration of the liberated material. The active substance was thermostable and appeared to have effects similar to those of synthetic plant growth stimulants. It has been demonstrated that salicylates were not responsible for the activity.

Introduction

Production of physiologically active materials by rooting cuttings* was a possibility drawn to the author's attention early in 1938. Liberation of growth-affecting materials by cuttings in the process of rooting is of both theoretical and practical interest, and may have a bearing on techniques of propagation. These considerations led to the initiation at that time of a series of experiments that were not taken beyond the preliminary stage. Some investigators have reported recently on plant growth inhibitors emanating from roots of actively growing guayule plants (2, 3). Others have observed possibly related phenomena (1, 5 to 8, 10). There is a possible relation between the active materials liberated by growing plants and those produced by rooting cuttings. Since liberation of active materials by plant cuttings has not been reported hitherto, communication of these results appears desirable.

Experimental

The investigation was confined to willow cuttings approximately 6 in. long, prepared from branches or whips of the current year's growth. Branches ranged in length from 18 to 24 in.; the cuttings therefrom were segregated as to size and position. Experimental groups contained equal numbers of terminal cuttings and other stem cuttings† from the several positions down the branch. Most of the work involved cuttings of *Salix alba* collected from trees growing by the Rideau River, Ottawa, Ont. However, some experiments involved *S. Bebbiana* and *S. discolor* [identification based on Sargent (9)].

[1] *Manuscript received December 7, 1944.*

Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa. N.R.C. No. 1290.

[2] *Biochemist.*

* *This suggestion was made by Dr. H. T. Güssow, at that time Dominion Botanist, Science Service, Dominion Department of Agriculture.*

† *Throughout the rest of the paper cuttings other than terminal ones will be referred to simply as stem cuttings.*

The physiologically active preparations used in these experiments were obtained from *S. alba* by maintaining approximately 50 cuttings in 2 in. of water in a litre beaker until branch roots appeared, usually a period of about two weeks. Cuttings were then removed and the water filtered. The filtrate was termed "used" water. For the preparation of "used sand", 15 cuttings were rooted in white quartz sand in 4-in. pots. After about two weeks rooting, cuttings were carefully removed to avoid contaminating the sand with root fragments.

Water cultures made use of groups, usually of from 10 to 15 cuttings, placed in 250 ml. beakers in which solution level was maintained at 2 in. by adding water. All experiments involved distilled water unless otherwise specified. Cultures were placed, in random order, on a laboratory bench in a position where they received diffused light of moderate intensity. The laboratory temperature was about 22° C.

Tests in sand were conducted in 4-in. pots, sunk to sand level in a greenhouse propagation frame supplied with a shade of factory cotton. Cuttings were planted to a depth of 2 in. in white quartz sand that had a pH of from 6.15 to 6.44 when moistened with distilled water, and a pH of around 7.8 when tap water was used. During the course of the experiments pots were watered daily with tap water. Treatment of sand with used water or chemical solutions involved initial application of 250 ml. of test solution. Greenhouse temperature was 25 $\pm$ 5° C.

Various concentrations of pure chemical were tested in order to compare their effects with those of the materials liberated during the rooting of willow cuttings. Salicylates, synthetic plant hormones, and nutrient salts were among the chemicals selected for this purpose. Salicylates were chosen since glucosides of salicylic acid are known to occur in the bark of *Salix* species. A detailed description of the treatments is given in Tables I to VIII. The vegetative material, collected over the period from March to November, 1938, also is described in the tables. Since most of the cuttings rooted, data were usually confined to counts of the number and length of root per rooted cutting. In Experiment 7 the rate of root initiation was determined and mean root length calculated. When experiments were adequately replicated, data were subjected to the analysis of variance procedure.

Results

Experiment 1

The number of rooted *S. Bebbiana* cuttings and total number of roots per group, as counted on three successive days, are given in Table I. While the level of rooting was low, certain effects are suggested. Culture in used water resulted in more rooted cuttings and better root development than occurred in the water controls. Boiling used water for 10 min. does not destroy its activity, which was still apparent at a one-tenth dilution. Improved rooting also followed the use of salicylic acid, particularly at the 0.1 and 1.0 p.p.m. levels.

TABLE I

SOLUTION CULTURE OF *Salix Bebbiana* CUTTINGS COLLECTED IN LATE MARCH AND IMMEDIATELY PLANTED IN GROUPS OF 11 CUTTINGS (EXPERIMENT 1)

Culture medium	No. of cuttings with roots			No. of roots per group of 11 cuttings		
	Days after initiation of experiment					
	10	11	12	10	11	12
Tap water	1	1	2	1	1	2
Tap water in which willow cuttings had rooted	3	5	6	4	6	7**
Boiled tap water in which willow cuttings had rooted	3	5	6	4	6	9
One part once-used water and nine parts water	3	4	4	4	5	5**
Distilled water	2	3	4	3*	4*	5*
Salicylic acid solution, 0.01 p.p.m.***	4	4	4	6	6	6**
Salicylic acid solution, 0.1 p.p.m.***	3	4	5	4	7	8**
Salicylic acid solution, 1.0 p.p.m.***	4	6	6	5	7	9**
Salicylic acid solution, 5.0 p.p.m.***	4	4	4	4	5	5**

* *Roots relatively short.*

** *Secondary or branch roots in evidence.*

*** *Parts of chemical per million parts of solution by weight.*

Experiment 2

Effects of various chemicals at several concentrations on rooting of *S. alba* cuttings are described by the data of Table II. Acetic acid solutions at the 1 and 5 p.p.m. levels appeared to result in reduction of root length but the 0.01 p.p.m. dosage tended to increase root number. Beneficial effects from the 1 p.p.m. salicylic acid treatment were indicated, and the two higher potassium salicylate levels suggested injurious effects on root length. Indolylbutyric acid was markedly damaging and vitamin B_1 had no effect. There was a suggested increase in root length associated with use of used water; however a decline in number of roots was indicated. The used sand led to a very marked increase in number and length of roots per rooted cutting. Talc treatment of willow cuttings appeared to have no appreciable effect but use of 1000 p.p.m. indolylacetic acid in talc was favourable.

Experiment 3

Number and length of roots of cuttings from solution cultures of *S. alba* are given in Table III. Root length of terminal cuttings was reduced in used water. A beneficial effect was suggested by culture in 1 p.p.m. salicylic acid with terminal cuttings though the effect just failed to attain the 5% level of significance. Substantially similar effects were shown by the data for stem cuttings. However the variability was too great to demonstrate statistically significant effects. The greater variability in the response of stem cuttings was probably related to the variation in diameter.

Results from propagation in sand are given in Table IV. The results of Expt. 3B in Table IV indicate effects of fresh and used sand on dormant and

TABLE II

SOLUTION AND SAND CULTURE OF *Salix alba* COLLECTED APR. 12 WITH 12 CUTTINGS TO THE GROUP (EXPERIMENT 2)

Culture medium	Solution concentration, p.p.m.	Number of roots per rooted cutting	Length of roots, mm. per rooted cutting
*Solution cultures**			
Tap water	—	6.6	205
Distilled water	—	7.5	201
Acetic acid	0.01	8.7	211
Acetic acid	1.0	7.2	179
Acetic acid	5.0	6.6	178
Salicylic acid	0.01	7.2	188
Salicylic acid	1.0	8.3	242
Salicylic acid	5.0	8.1	225
Potassium salicylate	0.01	7.8	204
Potassium salicylate	1.0	6.6	190
Potassium salicylate	5.0	6.6	174
Indolylbutyric acid	0.01	8.1	148
Indolylbutyric acid	1.0	5.8	16
Vitamin B_1	0.01	7.4	190
Vitamin B_1	1.0	7.7	191
Tap water in which willow cuttings had rooted	—	5.8	231
*Sand cultures***			
Fresh sand	—	3.7	47
Sand in which willow cuttings had rooted previously	—	6.2	193
Fresh sand, talc treated cuttings	—	4.4	60
Fresh sand containing 1000 p.p.m. indolylacetic acid in talc	—	7.5	163

* *Cuttings removed from solution culture seven days after planting.*

** *Cuttings removed from sand culture eight days after planting.*

TABLE III

ROOTING RESPONSES OF *Salix alba* IN SOLUTION CULTURE

Cuttings, from dormant whips collected Apr. 15, bases stored in water at 35° F., planted May 19 and removed eight days later (Experiment 3A)

(Four replicate groups of 17 cuttings were used, six terminal to 11 stem)

Culture solution	Terminal cuttings		Stem cuttings	
	No. of roots	Length of roots, mm.	No. of roots	Length of roots, mm.
	Per rooted cutting			
Tap water	9.5	246	10.1	417
Water in which willows had been rooted previously	9.3	208	10.6	372
Salicylic acid, 1 p.p.m.	8.9	273	9.4	387
Necessary difference, 5% level		28.1		59

TABLE IV

EFFECTS OF SAND IN WHICH WILLOW CUTTINGS HAD BEEN ROOTED ON SUBSEQUENT ROOTING OF *Salix alba*

Kind of willow cutting	Propagation medium	Number of roots per per cutting	Length of roots per cutting, mm.
Experiment 3B			
Non-dormant*	New sand	8.6	151
Non-dormant*	Sand in which willows had rooted	7.9	184
Dormant**	New sand	8.0	146
Dormant**	Sand in which willows had rooted	7.3	191
Experiment 3C			
Dormant***	Fresh sand	12.1	195
	Sand in which willows had rooted	12.1	528
	Necessary difference, 5% level		249

* *Collected just as buds were opening. Duplicate groups of 15 cuttings planted May 1 and removed eight days later.*

** *Described in Table III. Duplicate groups of 15 cuttings planted May 1 and removed eight days later.*

*** *Described in Table III. Four replicates of 17 cuttings planted May 19 and removed 11 days later.*

non-dormant cuttings. While there was little effect on root number, used sand suggested a decrease. However the used sand tended to increase the root length for both types of cutting. The results of Expt. 3C in the table and Fig. 1 show the very marked increase in root length that followed the use of sand in which willow cuttings had rooted previously.

Experiment 4

Effects of several concentrations of chemicals on the rooting of *S. alba* cuttings are given in Table V. After treatment with the growth stimulating chemicals, indolylacetic, indolylbutyric, and naphthylacetic acids, cuttings tended to show an increase in number and length of roots at lower concentrations. However, very markedly injurious effects were apparent at the 20 and 80 p.p.m. levels, but indolylacetic acid became harmful only at the 80 p.p.m. dosage. Salicylic acid had no marked effect over its entire range of concentration and did not appear to interact with indolylacetic acid.

Experiment 5

It is apparent from the data in Table VI that used sand brought about an increase in number of roots and a very great increase in root length of *S. discolor* cuttings. By comparison, the beneficial effects were no longer apparent when twice-used sand was tested. Application of used water resulted in a significant decrease in root length. Application of salicylic and indolylacetic acids led to reduction in root number and length. Results in sand that had previously contained rooting *Coleus* appeared to be substantially equal to those of the control groups.

TABLE V

Salix alba CUTTINGS COLLECTED APR. 28 AS BUDS WERE OPENING, TREATED IN CHEMICAL SOLUTIONS FOR 24 HR., AND PROPAGATED 12 DAYS IN SAND (EXPERIMENT 4)

(UNREPLICATED GROUPS OF 10 CUTTINGS)

Treatment	Concentration of chemical, p.p.m.	Number of roots per rooted cutting	Length of root, mm. per rooted cutting
Water treated control*	–	3.9	78
Indolylbutyric acid	1.25	4.8	95
	5.00	7.2	137
	20.00	3.8	38
	80.00	1.0	5
Indolylacetic acid	1.25	4.3	107
	5.00	5.7	150
	20.00	4.7	190
	80.00	3.9	44
Naphthylacetic acid	1.25	4.5	110
	5.00	5.8	135
	20.00	4.0	77
	80.00	1.9	9
Salicylic acid	1.25	3.2	76
	5.00	4.9	97
	20.00	3.7	92
	80.00	4.5	102
Indolylacetic and salicylic acids each at	1.25	4.5	79
	5.00	6.1	136
	20.00	3.3	92
	80.00	2.5	39

* *There were three control groups.*

TABLE VI

Salix discolor WHIPS COLLECTED APR. 15, BASES STORED IN WATER AT 35° F., CUTTINGS PREPARED JUNE 10, AND REMOVED FROM SAND CULTURE JULY 5 (EXPERIMENT 5)

(QUADRUPLICATE GROUPS OF 17 CUTTINGS EACH)

Treatment of sand media	No. of roots per rooted cutting	Length of roots, mm., per rooted cutting
Fresh sand	3.0	65.8
Sand in which one lot of willows had been rooted previously	4.9	131.6
Sand in which two lots of willows had been rooted previously	3.1	74.8
Sand wet initially with water in which *S. alba* had been rooted	3.6	27.1
Sand wet with 1 p.p.m. salicylic acid	2.5	11.1
Sand wet with 0.1 p.p.m. indolylacetic acid	3.4	29.1
Sand in which hormone-dust-treated *Coleus* had been rooted	2.8	61.6
Necessary difference, 5% level	1.1	37.0

PLATE I

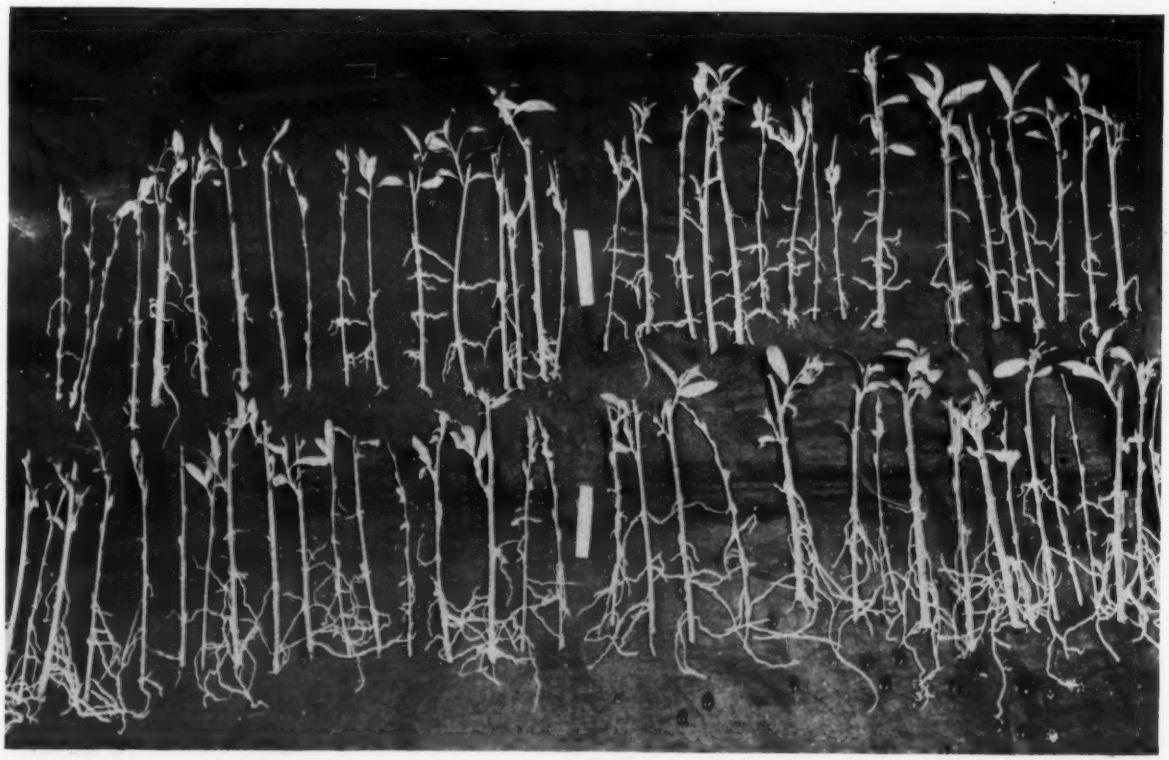

FIG. 1. *Salix alba cuttings propagated 11 days in sand. Upper: two groups from fresh sand. Lower: two groups from sand in which willow cuttings had rooted previously.*

Experiment 6

Data for the number of rooted and dead cuttings, and number and lengths of root per rooted *S. alba* cutting from a midsummer collection are given in Table VII. The main feature of the results was the reduced rooting and in-

TABLE VII

Salix alba CUTTINGS COLLECTED JULY 19 AND CULTURED IN SOLUTIONS FOR NINE DAYS (EXPERIMENT 6)

(TRIPLICATE GROUPS OF 17 STEM CUTTINGS EACH)

Culture solution	Percent of cuttings		Per rooted cutting	
	Rooted	Dead	No. of roots	Length of roots, mm.
Distilled water	18.8	57.2	9.0	47.0
Water in which willows had been rooted	6.8	89.2	8.5	15.3
Salicylic acid, 0.01 p.p.m.	16.0	69.2	6.8	16.3
Salicylic acid, 0.1 p.p.m.	16.0	68.0	7.2	37.8
Salicylic acid, 1.0 p.p.m.	18.8	61.2	6.6	37.8
Salicylic acid, 5.0 p.p.m.	20.0	65.2	6.7	27.2
Salicylic acid, 0.01 p.p.m. + 100 p.p.m. dipotassium hydrogen phosphate	22.8	60.0	9.2	67.3
Salicylic acid, 0.1 p.p.m. + 100 p.p.m. dipotassium hydrogen phosphate	16.0	72.0	10.0	34.6
Salicylic acid, 1.0 p.p.m. + 100 p.p.m. dipotassium hydrogen phosphate	17.2	69.2	8.0	45.6
Salicylic acid, 5.0 p.p.m. + 100 p.p.m. dipotassium hydrogen phosphate	14.8	73.2	12.0	55.6
Indolylacetic acid, 0.01 p.p.m.	14.8	60.0	8.2	41.7
Dipotassium hydrogen phosphate, 100 p.p.m.	13.2	58.8	9.5	34.7
1/100 Hoagland's nutrient solution	12.0	80.0	9.4	56.1
Necessary difference, 5% level	6.8	15.2		

creased mortality that accompanied the use of water in which cuttings had previously been rooted. There also was a suggestion of reduced root length. The used water treatment was significantly below all others with respect to effect on rooting, and above all others in relation to mortality, indicating that the concentration of the active materials attained was injurious. None of the other treatments increased the percentage of rooted cuttings above that of the controls, with dilute Hoagland's nutrient solution a possible exception. The combination of 5 p.p.m. salicylic acid and 100 p.p.m. dipotassium hydrogen phosphate resulted in greater mortality. Similar tests with terminal cuttings resulted in practically 100% mortality.

Experiment 7

It may be seen from Table VIII that used water caused a very great increase in the number of roots on dormant cuttings of a late autumn collection of *S. alba*. The average number of roots for such cuttings was 40.0 per group of 15; the average for all other treatments was 22.2. The stimulating effects of used water doubled the numerical rate at which roots were produced. Used water tended to reduce mean root length.

TABLE VIII

DORMANT *Salix alba* CUTTINGS COLLECTED AND CULTURED NOV. 9 (EXPERIMENT 7)
(QUINTUPLICATE GROUPS OF 15 CUTTINGS)

Culture solution	Average of daily root counts per group, taken over six days**	Final number of roots per group***	Mean root length, mm.***
Water control	5.3	18.2	23.6
Salicylic acid, 1 p.p.m.	6.7	15.8	27.6
Salicylic acid, 5 p.p.m.	7.6	29.4	26.9
Water in which willow cuttings had rooted	15.7*	40.0	19.1
Hydroquinone, 1 p.p.m.	5.4	21.2	26.1
Potassium dihydrogen phosphate, 100 p.p.m.	6.7	26.6	24.0
Necessary difference, 5% level		11.4	5.3

* *This value is very significantly above all others, which do not differ among themselves.*

** *Started Nov. 21. Actual data given; calculations of rates and necessary difference based on transformed data.*

*** *Determined Dec. 3.*

Discussion

Interpretation of these experiments necessitates recognition of the physiological variability of the test materials. Cuttings taken towards the close of the dormancy period, at the outset of new growth, during the summer, or in the late autumn show marked differences in their response to treatment. The active materials liberated may be presumed to vary both in amount and composition, depending on the time of collection of the source cutting. Bearing these complicating circumstances in mind, certain conclusions appear to be justified.

Dormant cuttings propagated either in used sand or used water tended to be beneficially affected in regard to rooting. The effects were particularly marked in the solution cultures of dormant cuttings collected in late autumn (Experiment 7). In these cuttings, rate of root initiation and root number were greatly increased by used water. Conversely, cuttings collected in the spring and treated with used water tended to be affected injuriously, especially with respect to root length. Once-used sand was beneficial, twice-used sand yielded results similar to those of the control, suggesting that the optimal concentration had been exceeded. Sand drenched with used water also was injurious. These results suggest concentration effects similar to those often encountered with synthetic plant growth stimulants (4).

The active material liberated during rooting of willow cuttings is thermostable, soluble in water, and capable of maintaining a large part of its activity for periods of at least three months. While the initial results suggested beneficial effects from salicylic acid these were confined to cuttings collected in the spring.

It is of interest to point to the similarity in effect of used sand and dust treatment of willow cuttings with 1000 p.p.m. indolylacetic acid. The response to plant hormone chemicals was conditioned by the physiological state of the cutting. These results suggest that substances substantially similar to synthetic plant growth stimulants are included in the active material from rooting willow cuttings. It is recognized that products of the activity of micro-organisms may have a bearing on these results but this has not been investigated.

In view of the indicative nature of the foregoing experiments the consideration of the liberation of physiologically active materials by rooting cuttings is one that may merit the attention of horticulturists, since it could have a bearing on the interpretation of propagation experiments.

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NOTES ON SEED-BORNE FUNGI

III. *CURVULARIA*[1]

By J. W. Groves[2] and A. J. Skolko[2]

Abstract

In the examination of agricultural seeds for the presence of seed-borne fungi, five species of *Curvularia* have been isolated. Three species, *C. geniculata* (Tr. & Earle) Boedijn, *C. inaequalis* (Shear) Boed., and *C. Trifolii* (Kauffm.) Boed., have been identified by comparison with types. Two species, *C. lunata* (Wakker) Boed., and *C. pallescens* Boed., have been provisionally identified from the literature. The types of four North American species, *C. geniculata* (Tr. & Earle) Boed. (*Helminthosporium geniculatum* Tr. & Earle), *C. inaequalis* (Shear) Boed. (*H. inaequalis* Shear), *C. Trifolii* (Kauffm.) Boed. (*Brachysporium Trifolii* Kauffm.), and *C. falcata* (Tehon) Boed. (*Acrothecium falcatum* Tehon), have been examined. *Helminthosporium Cymbopogi* C. W. Dodge belongs to this genus and is transferred here as *Curvularia Cymbopogi* (C. W. Dodge) n. comb. The species isolated from seeds do not appear to be of serious pathological significance.

The Genus *Curvularia*

The genus *Curvularia* was erected by Boedijn (2) to include a group of phragmosporous Dematiaceae with certain well defined characters in common. These characters may be summarized as follows:

1. The spores are borne spirally or in whorls.
2. The third cell from the base of the spore is disproportionately large.
3. The spores tend to be more or less strongly curved.
4. The end cells tend to remain more or less permanently hyaline.

Fungi exhibiting these characters have been described in several different genera and Mason (8) first attempted to bring them together under the generic name *Acrothecium*. He recognized that the type of *Acrothecium* was not well known and that the description did not fit these fungi in all respects. Boedijn (2) collected a fungus in which the spores were borne on the rounded or swollen end of the conidiophore rather than in whorls and he considered that this was closer to the type of *Acrothecium* than the fungi placed there by Mason. Accordingly he erected the genus *Curvularia* to include the species with spores borne in whorls or spirals.

Boedijn attempted to bring together the various species exhibiting the above characters that had been described under different genera and he listed a total of 18 species. He divided the genus into three sections termed the "maculans" group, the "lunata" group, and the "geniculata" group. The "maculans" group is characterized by having three-septate spores in which the two central cells are larger and darker than the two end cells and the spores are only slightly curved. The "lunata" group is characterized by

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having three-septate spores that are mostly strongly curved and have only one cell disproportionately large. The "geniculata" group is characterized by having spores with more than three septa.

In general the genus is not well known as indicated by the discrepancies in the descriptions given by different authors. Boedijn studied a number of species in culture, but many were only known to him from descriptions and frequently these were inadequate.

In the hope of contributing some additional information, a study was undertaken of the species of *Curvularia* that have appeared from time to time in the course of our examination of agricultural seeds for the presence of seed-borne fungi. The species encountered do not appear to exhibit marked host specificity nor do they seem to be of any pathological significance. The isolates obtained are apparently representative of five species, three in the "lunata" group and two in the "geniculata" group. In order to identify the isolates from seeds it has been necessary to review, as critically as possible, all of the species described in the "geniculata" and "lunata" groups. The types of four species and a subculture of one of the original isolations of a fifth species have been examined and, of the five species isolated, three have been identified by comparison with type material.

Cultural Characters

Bonar (3) has published a detailed study of *Curvularia Trifolii* (Kauffm.) Boed. in culture, and Shear (12), Bainier (1), and Boedijn (2) have also cultured species of this genus. In the isolates that we have obtained from seeds, no noteworthy differences have been observed, hence it is considered preferable to make some observations on the cultural characters in general rather than discuss them under each species.

On malt extract agar the cultures reach a diameter of 5 to 10 cm. in 10 days at room temperature. They are at first white with scant aerial mycelium in radiating tufts or strands. With the production of spores, which is usually copious, they soon darken, becoming dark olive-green to black. The colonies often superficially resemble those of *Helminthosporium sativum*. Slight variations may be observed in the rate of growth, amount of aerial mycelium, spore production, etc., but sectors of individual colonies show as much variation as different isolates in these respects. Sometimes the cultures produce hard, black, erect, cylindric, branched stromata (Fig. 7). These have been observed by other authors and have been described in detail by Bonar (3), but their taxonomic significance is obscure. In our cultures they appeared only rarely and sporadically and subsequent transfers did not always produce them again. It would seem that they are of little value as a criterion of species.

The "geniculata" Group

The species most commonly isolated from seeds fall into this group, which is characterized by having spores with more than three cross septa. The

following species with the dates of original publication were listed by Boedijn in the "geniculata" group:

C. geniculata (Tracy & Earle) Boed.	1896
C. spicata (Bainier) Boed.	1908
C. falcata (Tehon) Boed.	1919
C. fallax Boed.	1933
C. affinis Boed.	1933

To these must be added two species that Boedijn erroneously placed in the "lunata" group. Since he did not describe them he evidently had not seen any specimens.

C. inaequalis (Shear) Boed.	1907
C. ramosa (Bainier) Boed.	1908

Finally the species described by Dodge (4) as *Helminthosporium Cymbopogi* is a typical *Curvularia* belonging to the "geniculata" group and must be transferred to this genus.

Curvularia Cymbopogi (C. W. Dodge) n. comb.

Helminthosporium Cymbopogi C. W. Dodge. Ann. Missouri Botan. Garden, 29: 139. 1942.

C. geniculata (Tr. & Earle) Boedijn. Bull. Jardin Botan. Buitenzorg, Ser. III, 13 : 129. 1933.

Helminthosporium geniculatum Tracy & Earle. Bull. Torr. Club, 23: 207. 1896.

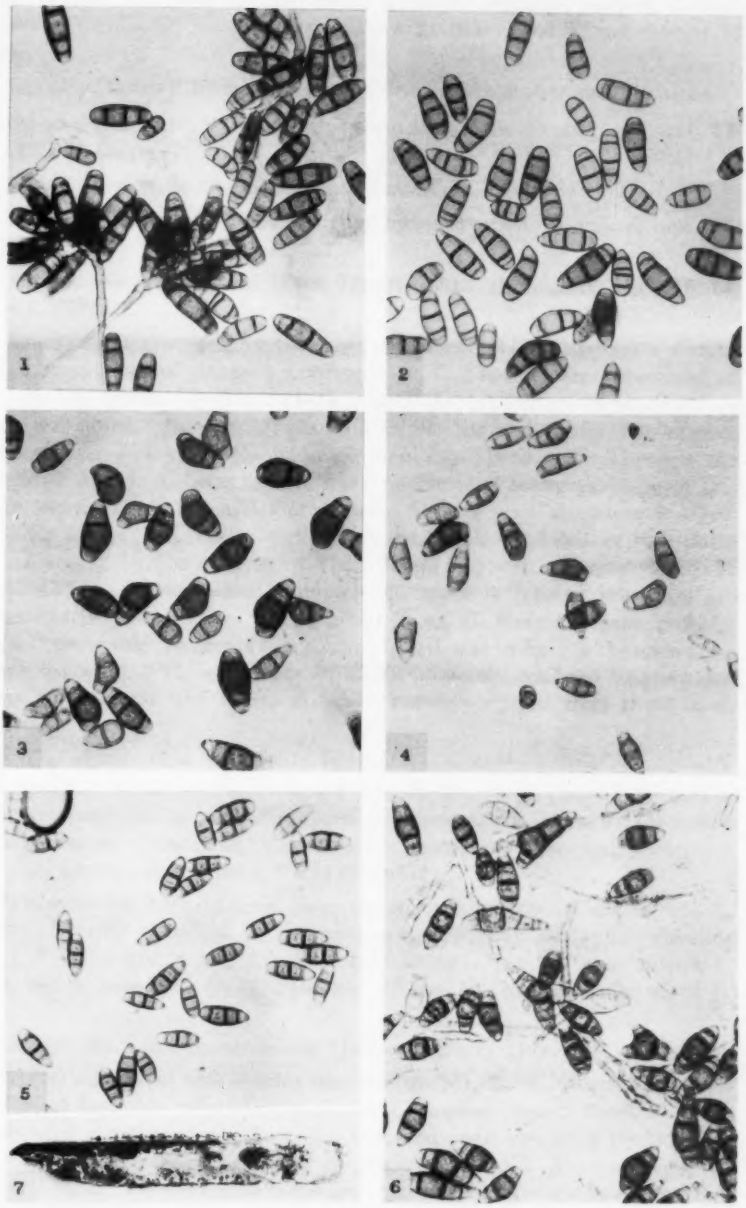
This species, which provides the earliest name in the group, was originally described as occurring on *Eragrostis rachitricha* and the spores were said to be 35–40 × 8–10 μ. Through the kindness of Mr. J. A. Stevenson it was possible to examine the type. This specimen consists of a fairly abundant collection of heads of *Eragrostis rachitricha*, but the fungus is not very plentiful and must be searched for carefully. The spores (Fig. 8) are brown, unequally ventricose-fusiform, mostly four-septate, the third cell from the base slightly larger and darker, and the end cells nearly hyaline, more or less curved to nearly straight, mostly not strongly curved, (21)–28–36–(42) × 11–14–(15) μ. The conidiophores are brown, septate, simple, geniculate at the tip, variable in length, (3)–4–5 μ in diameter. Thus we found the spores in the type specimen to be broader than stated in the original description.

Cultures have been isolated from seeds in which the spores agree closely in size and shape with this specimen. From the cultures the spores measure (21)–28–40–(50) × (10)–11–14–(15) μ (Figs. 1, 14).

EXPLANATION OF PLATE I

FIGS. 1 to 6. *Microphotographs of spores of Curvularia spp.* FIG. 1. *Curvularia geniculata (Tracy & Earle) Boedijn.* FIG. 2. *C. inaequalis (Shear) Boedijn.* FIG. 3. *C. Trifolii (Kauffman) Boedijn.* FIG. 4. *C. lunata (Wakker) Boedijn.* FIG. 5. *C. pallescens Boedijn.* FIG. 6. *C. Cymbopogi (Dodge) n. comb.* FIG. 7. *Cylindric stromata of C. pallescens, produced on malt agar slant.*

PLATE I



This species has been isolated from seeds of *Brassica oleracea* L. var. *capitata* L. (cabbage), *Linum usitatissimum* L. (flax), and *Pisum sativum* L. (peas).

It has been isolated from seeds from Ontario, Manitoba, and Michigan.

C. inaequalis (Shear) Boed. Bull. Jardin Botan. Buitenzorg, Ser. III, 13: 129. 1933.

Helminthosporium inaequalis Shear. Bull. Torr. Club, 34: 307. 1907.

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Curvularia ramosa Boedijn. Bull. Jardin Botan. Buitenzorg, Ser. III, 13: 129. 1933.

C. inaequalis was originally described by Shear (12), based on a fungus isolated from pulp of diseased cranberries. The spores were described as three- to five-celled, 22–32 × 11–14 μ and the production of stromata in culture was noted. Boedijn (2) placed it in the "lunata" group, but he gave no description and evidently had not seen any specimens. Through the kindness of Mr. J. A. Stevenson it was possible to examine the type of this species, consisting of two slides made from Shear's original culture in 1906. The slides are in good condition although the hyaline end cells of the spores have collapsed to some extent. The spores (Fig. 9) measure 27–35 × (11)–12–16 μ. An occasional three-septate spore is present but they are predominantly four-septate. They differ from *C. geniculata* principally in being a little broader in proportion to their length and in having the ends more obtusely rounded. The difference in width is mostly confined to the third cell and, as a result, the spores are more strongly curved than those of *C. geniculata*.

The *Curvularia* most frequently isolated from seeds agrees closely with *C. inaequalis*. In culture the spores are (24)–28–40–(60) × (10)–11–16–(18) μ, mostly four-septate, occasionally three- or five-septate (Figs. 2, 15). The conidiophores are brown, septate, simple or sometimes branched, geniculate at the tip, variable in length, 4–6 μ in diameter.

This species has been isolated from the following seeds: *Brassica rapa* L. (turnip), *Dactylis glomerata* L. (orchard grass), *Festuca elatior* L. (meadow fescue), *F. rubra* (red fescue), *Linum usitatissimum* L. (flax), *Pisum sativum* L. (peas), *Secale cereale* L. (rye), *Spinacia oleracea* L. (spinach), *Zea mays* L. (corn).

It has been isolated from seeds from Quebec, Ontario, Manitoba, and Illinois.

A typical culture of this species was sent to Mr. E. W. Mason, Imperial Mycological Institute, who identified it as *C. ramosa* (Bain.) Boed. by comparison with a culture from Australia that had been identified by Boedijn. *C. ramosa* was originally described by Bainier (1) as a *Brachycladium* on stems of *Urtica*. He studied it in culture and described the spores as 25–31 × 11–14 μ with three or four septa. He also noted the occurrence of stromata in

culture. There is nothing in the description to distinguish it from *C. inaequalis*. Boedijn (2) placed this species in the "lunata" group with no notes or description, indicating that he had not seen any material at that time. Since in our culture three-septate spores are rare and probably immature, and Mason found that the culture that he examined agreed well with the culture identified by Boedijn, this would suggest that Boedijn's concept of *C. ramosa* was based on work subsequent to his 1933 paper. On this basis then, *C. inaequalis* and *C. ramosa* are considered to be synonyms and since *C. inaequalis* is the earlier name it has been adopted.

We have separated *C. geniculata* and *C. inaequalis* on the basis of small differences in the spores. These differences are difficult to describe, but when mounts of the two fungi are compared they become apparent. The differences lie more in the proportion of length to width than in the absolute size of the spores. The lengths of the spores are much the same in both species, but in *C. geniculata* the width rarely exceeds 14 μ while in *C. inaequalis* they are frequently 15–16 μ in width. In an effort to express this difference, a minimum of 25 spores from each isolate was measured, a figure was obtained for each spore by dividing the length by the width, and the average of these figures was calculated. In *C. geniculata* the figure representing this average was 2.7 or 2.8, while in *C. inaequalis* it was usually 2.4, occasionally 2.5 or 2.3.

In mounts from some of the older cultures of *C. inaequalis* the spores were slightly smaller than in fresh cultures and the width scarcely exceeded 14 μ. However, the figure obtained as described above remained at 2.4, indicating that although the spore as a whole was smaller, the proportion of length to width remained the same.

In *C. inaequalis* the spores are usually more strongly curved than in *C. geniculata*, which would seem to be a result of the proportionately larger central cell. The shape of the basal cell is also slightly different in the two species. In *C. inaequalis* it is rounded and in *C. geniculata* it is more pointed.

The differences noted are not great but they do appear to remain constant and can be observed in the type specimens. It is, therefore, considered desirable to recognize both species, at least until more is known of their life histories that might justify uniting them.

C. spicata was originally described by Bainier (1) as a *Brachycladium* occurring on cow dung. He distinguished it from his *B. ramosum* by the lack of stromata in culture, and by the somewhat larger spores 40–42 × 11–14 μ borne in elongated heads rather than a few terminal whorls. It is clearly of

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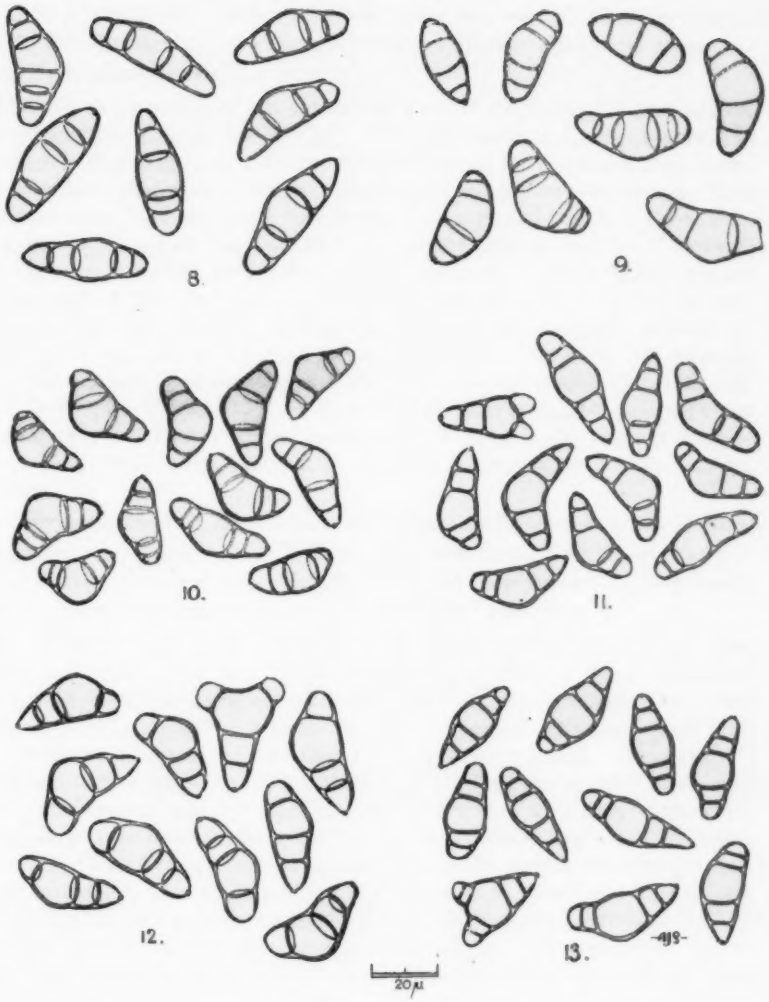
EXPLANATION OF PLATE II

Spores from type specimens (except Fig. 11). FIG. 8. *Curvularia geniculata (Tr. & Earle) Boed.* FIG. 9. *Helminthosporium inaequalis Shear.* FIG. 10. *Acrothecium falcatum Tehon.* FIG. 11. *Helminthosporium caryopsidum Sacc. (from Baker's Fungi Malayana 238).* FIG. 12. *Brachysporium Trifolii Kauffm.* FIG. 13. *Curvularia Cymbopogi C. W. Dodge.*


8.
9.
10.
11.
12.
13.
20μ

the "geniculata" group but nothing has been isolated from seeds that agrees with the description.

C. falcata[1] was originally described by Tehon (13) as an *Acrothecium* occurring on *Setaria*. In the original description the spores were stated to be 35 × 10 μ which would indicate a spore three and one-half times as long as broad, but the figures accompanying the description illustrated a proportionately broader spore.

Through the kindness of Dr. Tehon and Prof. N. E. Stevens it was possible to examine the type of this species. The type material is very scanty and although it does not agree with any species that we have isolated from seeds, it is considered desirable to put the results of our examination on record. The specimen consists of one head, four leaves, and a piece of culm. There were some conidiophores on the head and apparently also on one leaf. A mount was made from a little of the material on the head. The conidiophores are brown, septate, not branched, 3–6 μ in diameter, geniculate at the tip, and with the basal cell swollen. The spores (Fig. 10) are unequally ventricose-fusiform, mostly strongly curved or nearly straight, three- or four-septate but predominantly four-septate with the third cell larger and darker coloured, (22)–24–28–(31) × (10)–12–13–(14) μ. These spores agreed well with the original figures but not with the measurements given in the original description. They are somewhat similar to spores of *C. inaequalis* in shape but are smaller.

In connection with *C. falcata* two other species might be mentioned, *Helminthosporium caryopsidum* Sacc. and *H. curvulum* Sacc., both of which have spores of the *Curvularia* type. Boedijn excluded these species on the grounds that the types were mixtures of *C. lunata* and *C. geniculata*.

In the herbarium of the Division of Botany and Plant Pathology these species are represented only by the specimens in Baker's Fungi Malayana 238, labelled *H. caryopsidum*, and 239, labelled *H. curvulum*. In Fungi Malayana 217, labelled a mixture of *Clasterosporium maydicum* Sacc. and *H. curvulum*, no spores of the *Curvularia* type were found. Drechsler (5) published figures based on Fungi Malayana 239 illustrating both three- and four-septate spores and our material agrees very well with this. Mason (8) found only three-septate spores in another specimen of the same exsiccatus and stated that *H. caryopsidum* in 238 appeared to be morphologically identical to *H. curvulum*. Our specimen of 238 also has both three- and four-septate spores (Fig. 11) and appears to be morphologically indistinguishable from 239. Both of these specimens appear to be identical with the type of *C. falcata*. Since it is doubtful whether No. 239 can be regarded as the type of *H. curvulum* and No. 238 is definitely not the type of *H. caryopsidum* it would seem inad-

[1] *The original description gave the spelling "flacatum" for the specific name. This was evidently a typographical error for "falcatum" referring to the curved shape of the spores. On the type specimen the name is written "Acrothecium falcatum". Article 70 of the International Rules reads in part, "The original spelling of a name or epithet must be retained except in the case of a typographic error —".*

visable to unite these until they have been more critically studied. If they should prove to be identical, *H. caryopsidum* is the oldest name of the three.

C. fallax was isolated from the air by Boedijn (2). The spores were described as 23–36 × 9–14 μ and branched stromata were produced in culture. From the description it would appear to be very close to *C. inaequalis*.

C. affinis was also described by Boedijn (2) based on isolations from air, *Psidium*, *Eugenia*, and *Decaspermum*. The spores were said to be 25–36 × 8–12 μ, indicating a spore narrow in proportion to its length, and from the description it would seem that it was not strongly curved. Although no material of this species has been seen, the straighter, narrower spore is suggestive of *C. geniculata*.

C. Cymbopogi was described by Dodge (4) as a *Helminthosporium* causing a serious disease of citronella and lemon grass. In reply to a request for the loan of the type, Dr. Dodge kindly sent a transfer from one of his original cultures. The spores (Figs. 6, 13) in this culture measured (22)–27–35–(46) × (10)–11–13–(14) μ. This agrees well with the spores described by Dodge as occurring in his cultures but they are much smaller than he found them on the host. This is the only species in which this difference in size on the host and in culture has been noted. The spores in the culture that we examined are more strongly tapered toward the base than spores of *C. geniculata* which results in a slightly differently shaped spore although the size is close. Many of the spores exhibit peculiarities in shape, becoming forked or lobed to almost triangular. In view of these differences as well as the evident parasitic habit of this fungus, it would seem desirable to keep this species distinct at present.

The "lunata" Group

In the "lunata" group Boedijn described eight species of which two, *C. inaequalis* and *C. ramosa*, have been shown to belong to the "geniculata" group. The remaining six species with dates of original publication are listed below:

C. Crepini (Westend.) Boed.	1861
C. lunata (Wakker) Boed.	1898
C. Andropogonis (Zimmerm.) Boed.	1902
C. Trifolii (Kauffm.) Boed.	1920
C. Penniseti (Mitra) Boed.	1921
C. pallescens Boed.	1933

C. Crepini provides the oldest name in this group. Boedijn gave no description of this species, but Saccardo (11), Lindau (7), and Migula (9) give the spores as 50–60 × 17–20 μ, which is larger than any spores found in isolates from seeds.

EXPLANATION OF PLATES III AND IV

FIG. 14. *Curvularia geniculata (Tr. & Earle) Boedijn.* FIG. 15. *Curvularia inaequalis (Shear) Boedijn.*

PLATE III

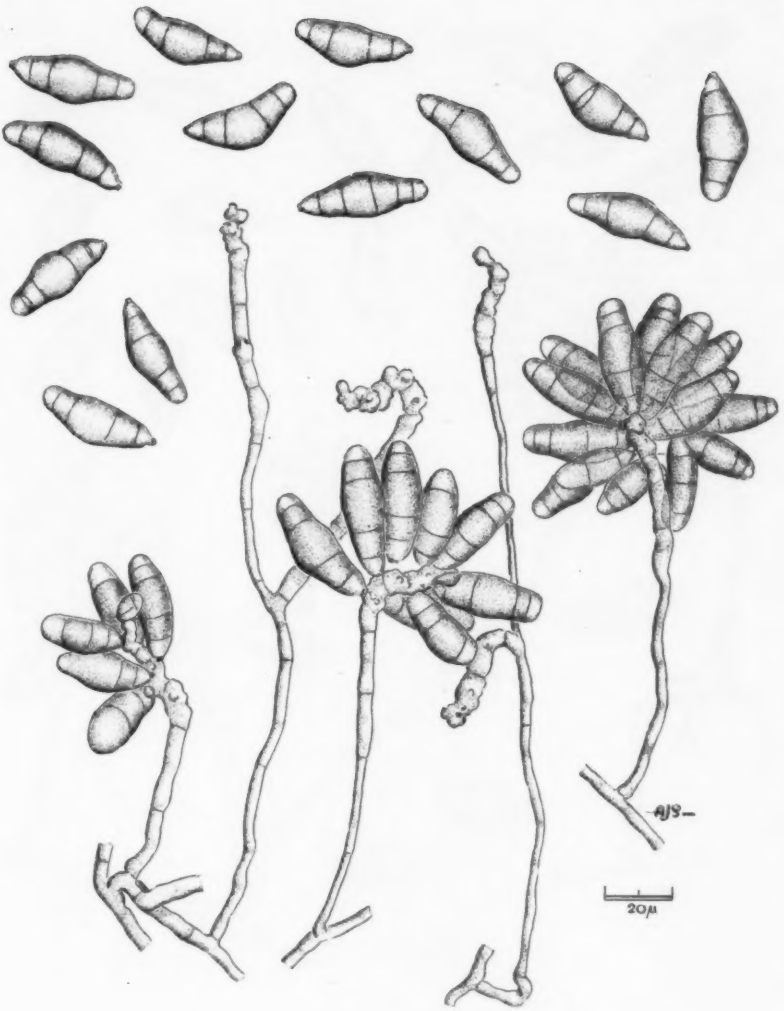


FIG. 14

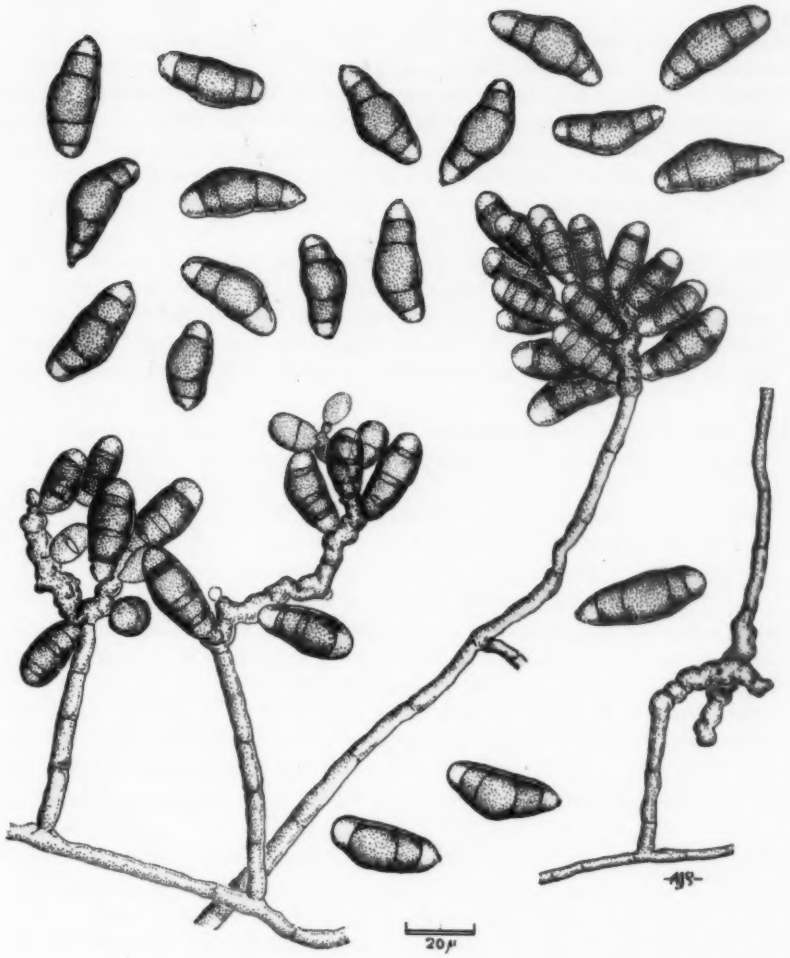


FIG. 15

C. lunata (Wakker) Boed. Bull. Jardin Botan. Buitenzorg, Ser. III, 13: 127. 1933.

Acrothecium lunatum Wakker. De ziekten van het suikerriet op Java. p. 196. Leyden. 1898.

C. lunata was originally described from Java on sugar-cane and the spores were said to be 30 × 10 μ. Mason (8), as a result of examining some isolates that he considered belonged to this species, suggested that the concept might be limited to forms with three-septate spores less than 12 μ in diameter. However Boedijn (2) had isolates from air, *Phalaenopsis*, *Ricinus*, and *Mangifera*, which he referred to this species and he described the spores as 19–30 × 8–16 μ. It is evident, therefore, that the concept of the species is not clear-cut.

It has not been possible to examine any authentic material of this species but we have referred to it two isolates that could not be otherwise disposed of. Among the published descriptions they seemed to come closest to Boedijn's concept of *C. lunata*, and presumably he had access to authentic material. No characters were observed in our isolates that would definitely exclude them from *C. lunata*.

In these isolates the spores (Figs. 4, 16) are pale brown, three-septate, the third cell from the base larger and darker coloured than the others, unequally ventricose-fusiform, more or less curved to nearly straight, (19.5)–22–28–(32) × (9)–10–13–(15.5) μ. The conidiophores are pale brown, septate, simple or branched, geniculate at the tip, variable in length, 3–5 μ in diameter.

The cultures were isolated from seeds of *Allium cepa* L. (onion) from California, and *Capsicum annuum* L. (pepper) from Michigan.

C. Andropogonis was originally described as a *Napicladium* on *Andropogon* and the spores were said to be 40 × 20 μ. Mason (8) pointed out that the width of the spores figured with the original description was less than half their length. Boedijn (2) gave the spore size as 38–66 × 13–23 μ and stated that the basal cell bore a small hyaline appendage. None of the isolates from seeds exhibit these characters.

C. Trifolii (Kauffm.) Boed. Bull. Jardin Botan. Buitenzorg, Ser. III, 13: 128. 1933.

Brachysporium Trifolii Kauffm. Phytopath. 10: 441. 1920.

C. Trifolii was originally described as a *Brachysporium* causing a disease of white clover. In the original description a *Blennoria* fructification that was considered to be a stage in the life history of the *Brachysporium* was described, but Bonar (3), who made a careful cultural study of the species, showed that the *Blennoria* was distinct.

Through the kindness of Dr. E. B. Mains it has been possible to examine the type material, which is, fortunately, abundant. The spores (Fig. 12) are brown, three-septate, the third cell larger and darker than the others, unequally ventricose-fusiform, mostly strongly curved, sometimes forked or triangular lobed, 25–35–(38) × 11–15 μ. The conidiophores are brown, septate, simple,

geniculate at the tip, 5–6 μ in diameter. This agrees well with the original description except for the diameter of the spores. The original description gave the diameter as 9–11 μ, but in the material that we examined from the type they were as stated above.

Cultures in which the spores agreed very well with those in the type of *C. Trifolii* have been isolated on a few occasions from seeds. They differ from the isolates referred to *C. lunata* in the darker brown, broader, and more strongly curved spores. In *C. lunata* the spores very rarely exceeded 14 μ in diameter, but in *C. Trifolii* they were frequently up to 16 μ and have been observed up to 19 μ. Also the disproportion in size of the third cell of the spores of *C. Trifolii* is usually more pronounced and there is a greater tendency to produce forked and triangular spores. In our cultures the spores (Figs. 3, 17) measure (20)–25–35–(38) $\times$ (10)–12–16–(19) μ. When a figure representing the average of the lengths of individual spores divided by the widths was calculated for this species as with *C. geniculata* and *C. inaequalis*, and compared with a similar figure calculated for *C. lunata*, it varied from 1.9–2.0 in both species indicating that the difference was in the absolute size of the spores rather than in their proportions.

This species has been isolated from seeds of *Glycine max* Merr. (soybeans) and *Pisum sativum* L. (peas) produced in Ontario, and from seeds of *Cucumis sativa* L. (cucumber) from New Jersey, and seeds of *Cucurbita Pepo* L. (pumpkin) from Connecticut.

The isolates from Leguminosae agreed very well with the type of *C. Trifolii* but the two isolates obtained from Cucurbitaceae are referred here with some hesitation. They do appear to be closer to *C. Trifolii* than to the isolates placed in *C. lunata* but they are somewhat intermediate in character. They have a greater proportion of smaller spores, many of which do not show the abruptly disproportionate increase in size in the third cell. However, the differences do not seem to be sufficiently clear-cut or great enough to justify the setting up of a new species and, therefore, we have referred them to *C. Trifolii* for the present.

C. Penniseti was described by Mitra (10) as an *Acrothecium* causing a disease of *Pennisetum* in India. The species was described after a cultural comparison with *C. lunata* and the spores were said to measure 30.4–41.8 $\times$ 17.1–20 μ. We have seen no specimens but the spores appear to be broader than any we have isolated. It is probably a distinct species.

C. pallescens Boed. Bull. Jardin Botan. Buitenzorg. Ser. III, 13 : 128, 1933.

This species was described by Boedijn based on an isolate he obtained from the air. It was characterized as having very pale brownish, three-septate

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EXPLANATION OF PLATES V TO VII

FIG. 16. *Curvularia lunata (Wakker) Boedijn.* FIG. 17. *Curvularia Trifolii (Kauffm.) Boedijn.* FIG. 18. *Curvularia pallescens Boedijn.*

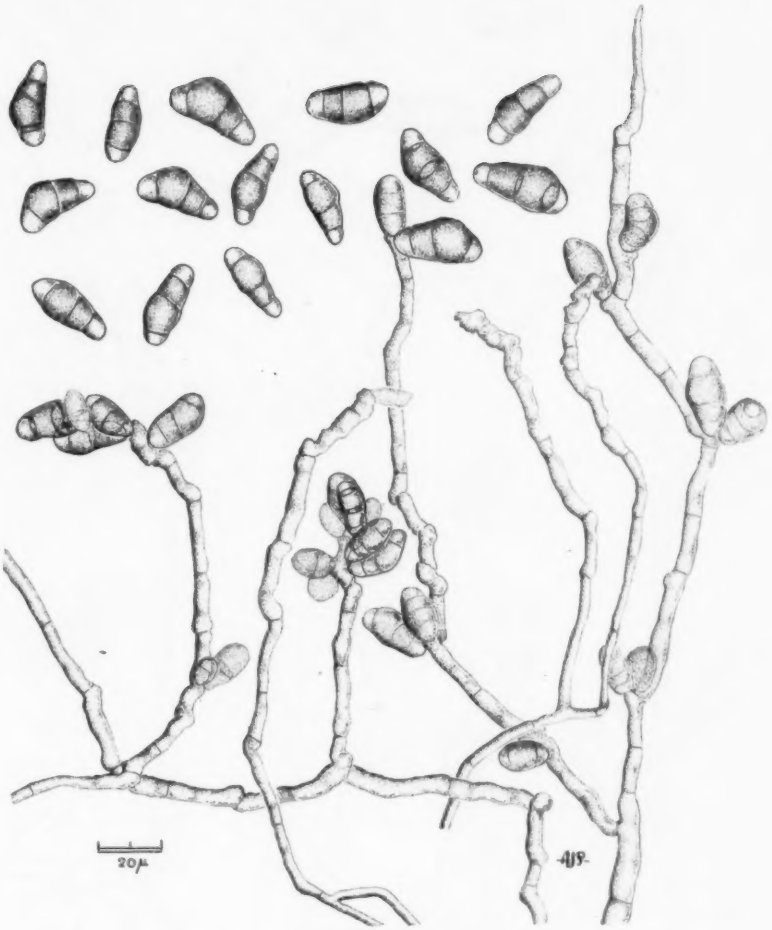

FIG. 16

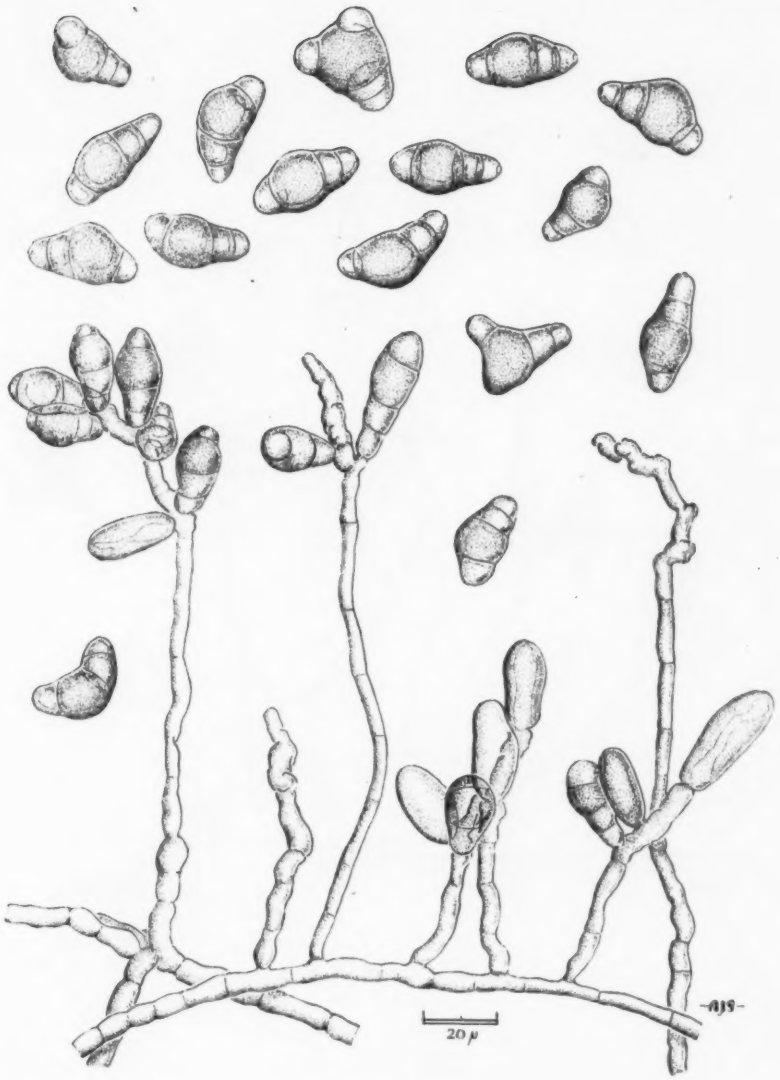

FIG. 17

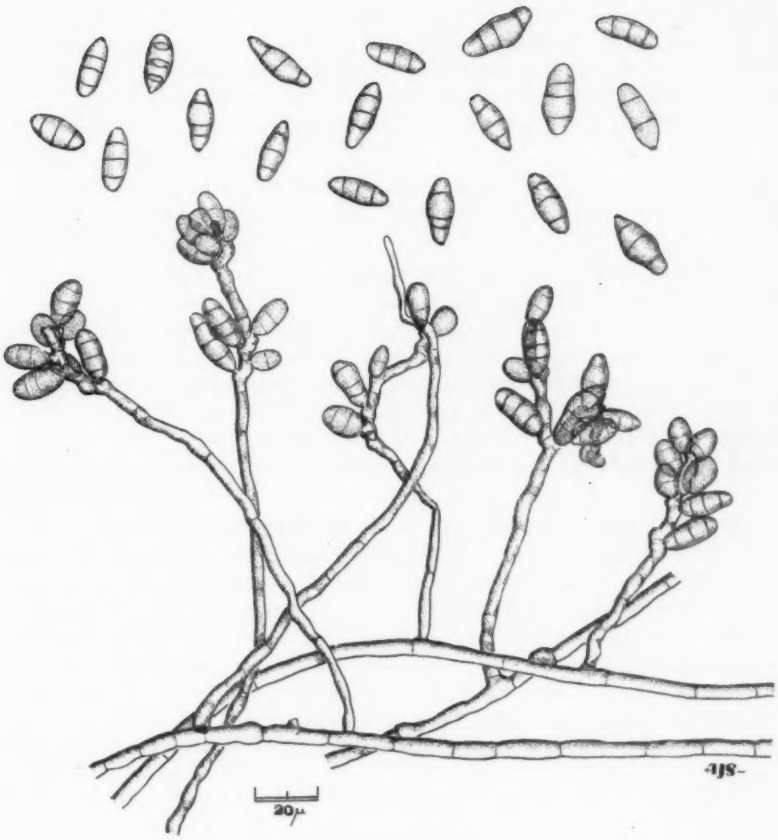

FIG. 18

spores, 21.5–30 × 7–11 μ, with the cell second from the top or the two central cells larger and darker.

We have referred two isolates to this species. In both of them there is copious development of cottony, greyish, aerial mycelium and rather sparse spore production. The spores are pale brown, three-septate, ellipsoid-fusiform to slightly unequally ventricose, the central cells slightly darker than the end cells, (15)–20–25–(30) × (7)–8–10–(11) μ. The third cell is not notably larger than the second, suggesting Boedijn's "maculans" group, but none of the species described in this group has spores as small as this, and from his description of *C. pallescens* it is evident that there was little difference in the two central cells. The conidiophores are pale brown, septate, simple, variable in length, 3–5 μ in diameter.

It has been isolated from seeds of *Brassica oleracea* var. *botrytis* L. (cauliflower) from British Columbia, and from seeds of *Brassica rapa* L. (turnip) from England.

It might be doubted whether a fungus isolated from the air in Java would be the same as one isolated from cauliflower seeds in British Columbia, and our identification might be questioned, especially as we have seen no authentic material of the Javanese fungus. However the appearance of the fungus a second time (on turnip seed from England) at least indicates its wide distribution. The English and Canadian isolates are indistinguishable. It is not known whether the fungus exhibits any specificity to Cruciferae or whether it is mere coincidence that both these isolates were obtained from cruciferous seeds.

It is remarkable among the isolates of *Curvularia* examined because of the small size of the spores. The figure obtained by taking the average of the lengths of individual spores divided by the widths was 2.4 which is greater than the corresponding ratios in any of the other representatives of the "lunata" group that have been isolated from seeds. The spore size fits Boedijn's description as does also the spore shape. In our present stage of knowledge of this group of fungi, the erection of a new species based solely on its occurrence on cruciferous seeds would seem to be unjustified. These isolates have, therefore, been tentatively referred to *C. pallescens*.

Conclusion

After studying a number of cultures of a particular group and coming to the conclusion that one of the most important characters in the recognition of species is the diameter of the spores, it is somewhat disconcerting to find, on examination of the type specimens, that they do not agree with the published descriptions on this critical point. We have examined the types of four species and a subculture of one of the original isolates of *Curvularia Cymbopogi* and, of these, only the culture had spores of which the size agreed with that stated by the original author. These discrepancies have been noted above in the accounts of the species concerned, but it seems worthwhile to

draw special attention to this point and to emphasize that had we been forced to rely solely on published descriptions in making our identifications, we should have been completely misled.

In a previous paper (6) we have expressed the view that in early studies of a little known group of fungi it is desirable to stress the differences when attempting to recognize species. As the fungi become better known, the value of these differences as specific diagnostic characters can be more accurately appraised and the species can be maintained or united. This would seem to cause less confusion than premature lumping of species that must be disentangled later, and accordingly in this account we have recognized species based on rather small differences in the spores. However these differences do seem to remain constant since some of our isolates have been grown in culture for more than three years.

This whole group is greatly in need of further study and it is scarcely possible at the present time to make definite statements regarding specific limitations. Of the 22 cultures that we have studied, we have identified four as *C. geniculata*, nine as *C. inaequalis*, five as *C. Trifolii*, two as *C. lunata*, and two as *C. pallescens*. Except for Boedijn's work little attempt has been made previously to make a comparative study of species of this group and the isolations from seeds have offered an opportunity to make a further contribution in this direction.

Acknowledgments

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